Resolution of budding yeast chromosomes using pulsed-field gel electrophoresis

Resolution of Budding Yeast Chromosomes using Pulse Field Gel Electrophoresis

Aziz El Hage 1 and Jonathan Houseley 2

1 Wellcome Trust Centre for Cell Biology, University of Edinburgh, Edinburgh, EH9 3JR, UK, aelhage@staffmail.ed.ac.uk

2 The Babraham Institute, Babraham Research Campus, Cambridge, CB22 3AT, UK, jon.houseley@babraham.ac.uk

Summary

Pulsed-field gel electrophoresis (PFGE) is a technique that resolves chromosome-sized DNA molecules in an agarose gel. As well as DNA mapping and karyotyping applications, PFGE techniques are well adapted to follow DNA rearrangements over time in a quantitative manner. Because of the very large sizes of the DNA molecules analysed, DNA preparation, electrophoresis and Southern blotting processes present unique challenges in PFGE experiments. In this chapter, we describe a robust PFGE protocol covering the preparation of intact *Saccharomyces cerevisiae* chromosomal DNA, specific running conditions for the resolution of small, medium- and large-sized chromosomes and their by-products, and basic Southern blotting and hybridization instructions for the analysis of these molecules.

Key words: *Saccharomyces cerevisiae*, pulsed-field gel electrophoresis (PFGE), contour-clamped homogeneous electric field (CHEF), agarose embedded yeast DNA, Southern blot, hybridization.

Running header: PFGE analysis of yeast chromosomes

1. Introduction

In conventional gel electrophoresis, DNA moves through the gel at a rate proportional to the logarithm of its size, leading to an exponential decline in resolution with increasing DNA length. This imposes a natural limit to the size of molecules that can be resolved up to the point where the DNA is so large that changes in length have no detectable effect on mobility. The best one-dimensional agarose gel electrophoresis methods have an effective resolution limit of ~50 kb in size, for which long running times and fragile low percentage gels are required. To circumvent the problems inherent to the resolution of large DNA molecules ingenious pulsed-field gel electrophoresis (PFGE) techniques were developed in the 1980s [1, 2], culminating in contour-clamped homogeneous electric field (CHEF) devices that are capable of reproducibly resolving molecules up to 12 Mb [3, 4].

During electrophoresis in agarose gels DNA molecules are "sieved" through the gel matrix in a size-dependent manner. Large linear molecules like DNA exist in solution as floppy coils, but are thoroughly unravelled as they squeeze through the much smaller pores in the agarose gel. Like a thread passing through the eye of the needle, the DNA is stretched out and therefore “reptates” (moves in a “snake-like” manner) through the gel. In PFGE techniques, instead of applying a constant electrical field as for conventional DNA electrophoresis, the direction of the electric field is periodically changed forcing the DNA molecules to rotate to align with the new field before progressing through the gel. Realignment occurs at a rate that decreases with the length of the molecule, so if the direction of the field is changed periodically (‘pulsed’), a larger molecule must spend more time realigning to each change of field than a smaller one, and will have less time to progress through the gel before the next field change. The realignment rate varies linearly with DNA length, ensuring that large and small molecules are resolved to a similar extent for a given change in size, avoiding the resolution limit in conventional DNA electrophoresis. In a standard CHEF protocol the direction of the electric field is switched between -60° and +60°, causing the DNA to zigzag through the gel while undergoing 120° angle changes [3]. Gels were originally run with a constant switching time, providing linear resolution within a given range, however because longer switch times resolve longer DNA molecules, switch times are now routinely increased through the run (‘ramped’), greatly improving the linear range of resolution [5].

PFGE methods have been traditionally applied to relatively simple tasks such as transgene mapping and karyotyping as well as analysing the products of recombination reactions. PFGE provides also a powerful quantitative method for following DNA rearrangements over time. Examples include formation and resolution of meiotic recombination intermediates [6] , damage and repair of chromosomes [7, 8], copy number changes [9, 10] and progression of DNA replication[11, 12].

DNA isolation by standard techniques is unsuitable for isolating intact chromosomes as pipetting and vortexing lead to shearing of long DNA molecules; even with very careful handling it is almost impossible to obtain DNA molecules larger than 100 kb. In order to avoid DNA shearing cell samples are lysed and the DNA is digested within a solid agarose plug. A large number of factors including running conditions and Southern blotting can also affect the quality of PFGE experiments. Here we describe in detail a robust PFGE protocol well suited for the separation of relatively small (as required for meiotic recombination analysis) and large DNA molecules (as required for ribosomal DNA recombination analysis). Careful following of this protocol should yield results of high quality and reproducibility.

2. Materials

Prepare all solutions using deionised water.

2.1 DNA preparation and gel running components

1. Agarose for DNA plug preparation (Seakem LE agarose, Lonza, catalogue number 50001) (*see* **Note 1**).

2. Plug Molds (Sample CHEF Disposable Plug Mold, Bio-Rad, catalogue number 1703706).

3. Lyticase (Sigma, catalogue number L2524) (*see* **Note 2**). Prepare 1 mL of Lyticase 17,000 U/mL stock by dissolving 17,000U lyticase powder (the activity concentration in U/mg is supplied by the manufacturer) in 490 µL water, 500 µL glycerol 100%, 5 µL 1M K_2HPO_4 and 5 µL 1M KH_2PO_4. Store at -20°C, stable for a few months.

4. Large orifice 200 µL tips (Starlab, catalogue number E 10118400).

5. Water bath (50°C) and heating block (37°C, 42°C, 50°C and 100°C).

6. Proteinase K (Roche, catalogue number 03115801001). Prepare 20 mg/mL solution in water. Store at -20°C.

7. Proteinase K buffer (PK buffer): 100 mM EDTA, 0.2% sodium deoxycholate and 1% N-laurylsarkosine sodium. Prepare fresh on day of use.

8. Wash buffer: 10 mM Tris-HCl pH 7.6 and 50 mM EDTA.

9. Gel rocker.

10. Certified Megabase agarose (Bio-Rad, catalogue number 161-3109).

11. Chromosome size marker: for low to medium range we use Midrange II PFG marker (NEB, catalogue number N3552S) or lambda ladder PFG marker (NEB, catalogue number N0340S), and for high range we use *H. wingei* chromosomes (Bio-Rad, catalogue number 170-3667).

12. TBE buffer: 0.5X or 1X depending on run conditions (see Table 1), prepared fresh (*see* **Note 3**). To make 2.5 L of 1X TBE: 27 g Tris base, 13.25 g boric acid, 10 mL 0.5 M EDTA pH 8. To make 2.5 L of 0.5X TBE: 13.5 g Tris base, 6.6 g boric acid, 5 mL 0.5 M EDTA pH 8.

13. PFGE machine: CHEF-DRII or DRIII System with chiller unit and casting stand (Bio-Rad, *see* **Note 4**).

14. SYBR Safe DNA gel stain (Molecular Probes, Invitrogen, catalogue number S33102) or Ethidium Bromide (10 mg/mL in water).

2.2 Southern blotting and hybridization components

1. Blotting membrane: positively charged nylon transfer Hybond-N^+ membrane (GE Healthcare, catalogue number RPN 303B)

2. Blotting paper: Whatman 3MM

3. Blotting solutions:

- depurinating solution - 0.25 M HCl (freshly diluted from concentrated HCl, usually 37% / 12.1 M);

- denaturing solution- 1.5 M NaCl, 0.5 M NaOH;

- neutralising solution - 0.5 M Tris-HCl pH 7.5, 1.5 M NaCl.

4. Paper towels for blotting (C-Fold hand towel 2 ply white, Scientific Laboratory Supplies, catalogue number FC 5804).

5. Transfer buffer (20X SSC): 3 M NaCl, 0.3 M Sodium Citrate, pH 7.0.

6. UV-crosslinking box (UV Stratalinker 1800, Stratagene).

7. Phenol:chloroform:isoamylalcohol (25:24:1) (PCI) (*see* **Note 5**).

8. 1.5 mL tight-cap (Safe-Lock, Eppendorf, catalogue number 0030 120.086) and 2 mL screw-cap (Anachem, catalogue numbers 2330-00 and 2001-52) microcentrifuge tubes.

9. 425-600 µm glass beads (Sigma, catalogue number G8772).

10. Break buffer: 10 mM Tris-HCl pH 8, 2% Triton X-100, 1% SDS, 100 mM NaCl and 1 mM EDTA.

11. 1X TE solution plus RNAse A: 10 mM Tris-HCl pH 8, 1mM EDTA and 0.25 µg/µL RNAse A (RNase A DNase-free, AppliChem, catalogue number A3832).

12. QIAquick Gel Extraction Kit (Qiagen, catalogue number 28704).

13. DECAprime II Random Primed DNA labelling Kit (Ambion, Invitrogen, catalogue number AM1455).

14. Liquid nitrogen (*see* **Note 6**).

15. [α-^{32}P]dATP or [α-^{32}P]dCTP (3000 Ci/mmol, 10 mCi/mL) (Perkin Elmer).

16. Mini Quick Spin DNA columns (Roche, catalogue number 11814419001).

17. Church Hybridization Buffer: 0.5 M sodium phosphate pH 7.2, 1 mM EDTA, 1% BSA and 7% SDS.

For preparation of 500 mL Church buffer: Dissolve 5 g BSA (bovine serum albumin fraction V) by small amounts in 100 mL water with stirring. In a separate 1 L beaker, mix 171 mL of 1 M Na_2HPO_4 with 79 mL of 1M NaH_2PO_4. Add 1 mL 0.5 M EDTA pH 8 and water to ~350 mL then 35 g SDS. Stir and warm gently to

dissolve then add water to 400 mL total volume. Add the BSA solution slowly with stirring then filter sterilize. Store at room temperature for short term, or aliquot and store at -80°C for long term.

18. Hybridization bottles, nylon mesh (VWR, catalogue number BARN308-6A) and rotary hybridization oven.

19. 10 or 20% SDS stock solution.

20. Phosphor Storage Screen with Cassette and PhosphorImager.

3. Methods

3.1 Preparation of agarose embedded yeast DNA

1. Weigh 0.16-0.2 g Seakem LE agarose in a 50 mL conical tube and add 10 mL dH_2O, seal and place in a beaker containing ~200 mL water (*see* **Note 7**). Bring agarose to the boil using a microwave then equilibrate to 50°C in a water bath.

2. Harvest 0.25-1 $x10^8$ cells (1.25-5 OD_{600}) from an appropriate growth phase by centrifuging at 2,000 g 5 min at 4°C (*see* **Note 8**). Re-suspend cells in 1 mL PFGE wash buffer, transfer to a microcentrifuge tube and spin again. Decant the supernatant and re-suspend cells in 50 µL wash buffer (*see* **Note 9**).

3. Warm cell suspension to 50°C in a water bath. Just prior to mixing the cells with agarose, add 1 µL lyticase and immediately proceed with step 4.

4. Combine cell suspension with an equal volume of agarose (from step 2) using a large orifice 200 µL tip. Mix by pipetting or vortex for 5-10 s and transfer the mixture to a plug mold (*see* **Note 10**). Let agarose polymerize.

5. Use the snap off tool provided on the plug mold to push the solidified agarose plug into a microcentrifuge tube containing 500 µL of wash buffer with 10 µL lyticase. Incubate plugs for 1 h at 37°C in a heating block.

6. Remove the wash buffer. Add 0.5 mL of PK buffer containing 1 mg/mL proteinase K. Incubate the plugs overnight at 50°C in a heating block.

7. Wash the plugs four times for 30 min each wash in 1 mL wash buffer, at room temperature with gentle agitation on a rocker. Store the plugs at 4°C in PFGE wash buffer or 0.5 M EDTA (*see* **Note 11**).

3.2 Casting the gel and loading the DNA plugs

Casting the gel requires the following components provided with the CHEF system: a casting stand with removable end gates, a platform on which the gel is cast and a comb (positioned on a comb holder).

1. Assemble the casting stand (*see* **Note 12**) and place the comb holder into one of the two positioning slots on each side of the casting stand. Make sure the bottom of the comb is approximately 1 mm above the surface of the platform. Prepare 120 mL of pulse-field Megabase agarose solution in TBE (*see* **Table 1** for recommended gel percentages and TBE concentrations) by microwaving, and ensure the agarose is fully dissolved (*see* **Note 13**). Cool to 60°C. Pour molten agarose into the casting stand and let solidify at room temperature.

2. Remove the comb holder gently. Fill the wells with buffer from the tank (*see* **Note 14**). Using clean tweezers place the DNA plug on a smooth surface e.g. a Petri dish and cut the plug in half with a clean razor. Load one half of the plug into the sample well (*see* **Note 15**). Include a chromosome size marker preferably in the first well of the gel.

3.3 Electrophoresis

1. Remove the gates from the casting stand. Place the gel and platform assembly in the frame in the centre of the electrophoresis cell (*see* **Note 16**).

2. Program the CHEF system with appropriate run conditions (*see* **Table 1** for example conditions). Start the run and make sure that the buffer is circulating through the cooler set to 12°C (*see* **Note 17**).

3.4 Gel staining and imaging

1. When the run is finished (*see* **Note 18**) place the gel in 200 mL 1X TBE with 1X SYBR Safe or 10 µg/mL Ethidium Bromide, in a plastic box.

2. Allow the gel to stain for 1 h with gentle agitation.

3. Wash two times 15 min each wash with 1x TBE to reduce the staining background.

4. Image the gel (*see* **Note 19**, example gel images are shown in **Figure 2**).

If needed process the gel for Southern blotting (steps 3.5-3.6).

3.5. Non-electrophoretic DNA transfer

1. Place the gel in a plastic box on a shaker with gentle agitation. Cover the gel with a solution of 0.25 M HCl and leave for 20-30 min to depurinate the DNA (*see* **Note 20).**

2. Discard the depurinating solution and soak the gel in the denaturing solution for 30 min to unwind the DNA strands and introduce breaks at the abasic sites generated by depurination. This will assist DNA transfer by breaking long DNA molecules into small pieces.

3. Discard the denaturing solution and soak the gel in the neutralizing solution two times 15 min.

4. Cut a piece of Hybond membrane the same size as the gel. Wet with water then soak with 20X SSC. Cut two pieces of blotting paper the same size as the gel, and one piece the same width as the gel but ~50cm long. Soak all blotting papers in 20X SSC.

5. Set up the capillary transfer as shown in Figure 1. Fill the plastic box (24 cm x 24 cm x 7 cm) with ~1 L 20X SSC and balance a long glass plate on top. Drape the long sheet of blotting paper over the glass plate, touching the liquid on each side to form a wick, and place a sheet of blotting paper on top. Place the gel on the blotting papers then carefully lay the membrane on the gel (*see* **Note 21**). Place a sheet of blotting paper on the membrane then role a pipette over the assembly to remove bubbles. Cover exposed areas of the wick with Saran Wrap or Parafilm to avoid short circuits, then add a stack of paper towels, a glass plate and a weight.

6. Transfer the DNA for at least 24 h.

6. Disassemble the transfer setup: carefully pull the membrane off the gel and place it on a sheet of dry blotting paper so that the surface with the bound DNA is facing upwards. Place the membrane inside the Stratalinker and immobilize the DNA to the membrane by UV crosslinking (120 mJ/cm^2).

3.6. Southern hybridization

1. Prepare the template for random primed probe synthesis in step 3.6.4. It should be a DNA fragment of 0.2-1 kb derived by PCR from yeast genomic DNA (step 3.6.2) or by restriction digest of a plasmid (skip step 3.6.2).

2. If yeast genomic DNA is required as a PCR template: break a yeast cell pellet (2-5 mL overnight saturated culture) in the presence of 200 µL break buffer, 200 µL PCI and 200 µL glass beads in a screw-cap tube, by vortexing for 5-10 min at room temperature. Centrifuge at room temperature for 10 min 20,000 g (full speed in a microcentrifuge). Remove carefully ~140 µL from the upper-phase and precipitate DNA with 0.5 mL of 100% ethanol. Spin in a microcentrifuge at full speed for 10 min at 4°C, discard the supernatant and wash the DNA pellet with 0.5 mL of 70% ethanol. Centrifuge at full speed for 10 min at 4° C, discard the supernatant, dry the DNA pellet and re-suspend it in 25-40 µL of 1X TE solution plus RNAse A . Incubate 10-15 min at 42° C to degrade the RNA. Set-up a standard PCR reaction using 1 µL of 1/10 dilution of the genomic DNA

3. Load the PCR or restriction digest on a standard 1% agarose gel stained with 1X SYBR Safe or 10 µg/mL Ethidium Bromide, run until your band of interest is in the middle of the gel, cut the DNA band and purify using QIAquick Gel Extraction kit. Elute the DNA from the QIAquick column with 30µL water.

4. Synthesise a random primed probe using DECAprime II Random Primed DNA labelling Kit following the manufacturer's procedure (*see* **Note 6**). You can use either [α-32P]dATP or [α-32P]dCTP (3000 Ci/mmol) (*see* **Note 22**).

5. Meanwhile, place the membrane from section 3.5 in a hybridisation bottle with 10 mL Church buffer (*see* **Note 23**). Attach the bottle to the rotisserie of a hybridisation oven set to 65°C and balance with an empty bottle if needed. Pre-hybridize at 65°C for at least 1 h while rotating.

6. Purify the probe using a Sephadex G50 based column. Ensure that more activity is present in the flow-through than remains on the column (this can be assessed by holding the column or tube a fixed distance from a Geiger counter using tweezers). Discard the column, the purified probe is in the flow-through.

7. Denature the radio-labelled probe by heating at 100°C for 10 min, snap chill on ice and add to the hybridization bottle. Hybridize for 12-24 h at 65°C with rotation.

8. Pour off the probe (*see* **Note 24**). Wash the membrane two times 10 min in 2X SSC, 0.1% SDS at 65°C and two times 20 min in 0.5X SSC, 0.1% SDS at 65°C (*see* **Note 25**).

9. Wrap the membrane in Saran Wrap and place in a cassette with a phosphor storage screen.

10. Scan with a PhosphorImager and if necessary perform longer exposures (*see* **Figure 2** for example results).

11. You can strip the ^{32}P-labelled probe by placing the nylon membrane in a plastic box containing a boiling solution of 0.1X SSC, 0.1% SDS. Continuous shaking at room temperature on a rocker helps the stripping process. Repeat the procedure several times until the radioactive signal disappears completely. You can re-probe the membrane as many times as you wish providing that the stripping is successful (see **Note 26**).

4. Notes

1. Although most PFGE protocols require low melting point agarose, in our hands plugs made using SeaKem LE perform equivalently and are much easier to handle.

2. Contaminants in lower grades of lyticase affect PFGE resolution.

3. We have had variable results with TBE diluted from 10X stocks. We therefore make fresh 1X/0.5X each time.

4. Bio-Rad CHEF systems are by far the most widely used PFGE systems, and are compatible with the running conditions described here. PFGE units are available without controlled refrigeration units, however PFGE runs capable of separating chromosome-sized molecules take 24 h or more at relatively high electrical field, and must therefore be carried out with precise temperature control to avoid gel distortions.

5. Use phenol pre-equilibrated with 10 mM Tris-HCl pH8, 1 mM EDTA (Sigma, catalogue number P4557).

6. Liquid nitrogen will be used in step 3.6.4 for random primed probe synthesis. Note that tight-cap tubes should be used to avoid bursting in liquid nitrogen.

7. Microwaving the conical tube in a small beaker containing water prevents the agarose from boiling too vigorously.

8. For the simplest analysis, use stationary cells grown 1-2 days in YPD. For time-dependent processes such as replication, DNA damage or recombination, cells should be in mid-log or other appropriate phase (e.g. meiosis). Note that cells can be fixed by re-suspending in 70% ethanol and stored at -20°C for later processing if desired.

9. Cells that flocculate (e.g. SK1 strain background) should be pipetted repeatedly until re-suspended at this point.

10. It is advantageous to place the molds at 4°C to rapidly solidify the agarose plugs.

11. The plugs are stable at 4°C for at least a year.

12. Place the platform into the casting stand. Position one end gate over the screws protruding from the casting stand, insuring that the horizontal slot is facing towards the platform. Tighten the screws. Similarly, position the second end gate and tighten the screws. See the CHEF system manual (Bio-Rad) for diagrams. Use a spirit level to make sure the platform is sitting on a level surface. The casting stand provided with CHEF-DR systems is 14 cm wide x 13 cm long.

13. We usually prepare 2.5 L TBE (0.5X or 1X, Table 1), use 120 mL for the agarose gel, and pour the remaining in the electrophoresis cell. Both the pump and cooler should be switched on at this stage to allow the buffer to equilibrate to 12°C.

14. Adding buffer in the wells helps avoiding air bubbles being trapped between the plug and the wall of the well sample.

15. Handling agarose plugs with tweezers requires practice; too tight a grip will squash the plug, but plugs are easily dropped and lost with tentative handling. The plug is often a little larger than the well but can easily be

squeezed in the well. If the plug is much smaller than the well ensure that it sits against the front edge of the well.

16. Make sure not to dislodge the gel from the platform otherwise the gel will float away during the run, obviously ruining the migration of the DNA molecules. Also ensure that the wells of the gel are facing the correct end (this information may change on different systems – check the CHEF system manual).

17. Because of the use of ramped switch times, optimisation of novel PFGE run conditions is complex and beyond the scope of this review. The easiest approach is to use the conditions recommended for a PFGE marker that gives resolution in an appropriate range for your application. Alternatively, run conditions can be sourced [13] from publications, and three widely applicable run conditions are given here in **Table 1**.

18. After each run, it is important to drain the running buffer from the electrophoresis cell and to allow circulation of 2.5 L distilled water for at least 30 min to ensure proper washing of the cell and the tubing.

19. Standard gel imaging systems can struggle to obtain good pictures of yeast PFGE gels as the staining intensity is often low. Laser scanning systems (we use FLA-5100 and FLA-7000 systems from FUJI / GE life sciences) provide much better results.

20. It is important to depurinate the DNA for at least 20 min and not more than 30 min otherwise the yield or quality of the transferred DNA can be greatly reduced.

21. Do not move the membrane after it has touched the gel. Small amounts of DNA transfer immediately, causing faint extra bands to appear on hybridization if the gel is moved.

22. Manipulate the radioisotope in a radiation protected area.

23. Hybridisation mesh can be used if desired - this can help ensure even signal on large membranes.

24. The hybridization mix can be stored in a 50 mL conical tube at -20°C for subsequent Southern hybridizations. Make sure to heat-denature the probe in a boiling water bath for at least 10 min before each use.

25. If washes have been performed in hybridization bottles, some background activity may be present where the membrane overlapped. To remove this background, wash the membrane for ~1 h with 0.1X SSC, 0.1% SDS in a plastic box at room temperature.

26. Note that for repetitive regions like the rDNA (150-200 copies in yeast) it is not trivial to strip the probe from the membrane. Therefore, if a blot is to be probed for sequences of different copy number, we recommend that you probe for lower copy targets first.

Acknowledgements

We thank David Barrass for critical reading of the manuscript. The set-up of the PFGE protocol was initially started in the laboratory of Professor David Tollervey.

AEH is a research fellow in the laboratory of Professor David Tollervey who is funded by the Welcome Trust. JH is funded by Wellcome Trust grant 088335.

Figure Captions

Fig.1. *Schematics of a basic Southern blotting assembly.*

Fig.2. *Examples of separation of large-, medium and small-sized chromosomes with PFGE.* In all panels, gel images are shown on the left and Southern blots on the right.

A. Separation of chromosome XII with large-sized running conditions. The length of chr. XII varies depending on the copy number of ribosomal DNA (for an rDNA array of 150-200 repeats chr. XII size can vary between 2.36-2.86 Mb). In some yeast mutants, here double *trf4*Δ *fob1*Δ strain [13], the rDNA array can be contracted to as little as 35 repeats (chr. XII size ~1.37 Mb). The probe on the Southern blot (right panel) is specific to the 18S rDNA.

B. Crude mapping of a randomly inserted subtelomeric transgene using medium-sized running conditions. A probe to the inserted NAT cassette (right panel) displays transgenes on different chromosomes.

C. Separation of meiotic recombination intermediates in a *sae2*Δ strain [14] using small-sized running conditions. Most chromosomes co-migrate as a single strong band near the top of the gel, whilst the three smallest - chr. I, VI and III resolve in the gel at 225 Kb, 295 Kb and ~350 Kb (left panel). A probe to the *MRC1* gene, which is located on chr. III, displays the full-length chromosome in all lanes and truncated DNA fragments (40-300 Kb) accumulating during meiosis (right panel).

MW= Molecular weight; M= chromosome marker

Table Captions

Table 1: *PFGE running conditions for three ranges of DNA,* see example results in Figure 2.

Formatted: Font: Not Italic

Table 1

Molecular weight range resolved	**Small 40 to 400kb**	**Medium 200 to 1500kb**	**Large 1000 to >3500kb**
Gel percentage	1.3%	1%	0.8%
TBE	0.5X	0.5X	1X
Run time	24 h	24 h	68 h
Switch times (ramped)	15-25s	60-120 s	300-900s
Voltage	6V/cm	6V/cm	3V/cm

References

1. Carle, G.F., and Olson, M.V. (1984). Separation of chromosomal DNA molecules from yeast by orthogonal-field-alternation gel electrophoresis. Nucleic Acids Res *12*, 5647-5664.
2. Schwartz, D.C., and Cantor, C.R. (1984). Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell *37*, 67-75.
3. Chu, G., Vollrath, D., and Davis, R.W. (1986). Separation of large DNA molecules by contour-clamped homogeneous electric fields. Science *234*, 1582-1585.
4. Orbach, M.J., Vollrath, D., Davis, R.W., and Yanofsky, C. (1988). An electrophoretic karyotype of Neurospora crassa. Mol Cell Biol *8*, 1469-1473.
5. Clark, S.M., Lai, E., Birren, B.W., and Hood, L. (1988). A novel instrument for separating large DNA molecules with pulsed homogeneous electric fields. Science *241*, 1203-1205.
6. Game, J.C. (1992). Pulsed-field gel analysis of the pattern of DNA double-strand breaks in the Saccharomyces genome during meiosis. Dev Genet *13*, 485-497.
7. Choy, J.S., and Kron, S.J. (2002). NuA4 subunit Yng2 function in intra-S-phase DNA damage response. Mol Cell Biol *22*, 8215-8225.
8. Lundin, C., North, M., Erixon, K., Walters, K., Jenssen, D., Goldman, A.S., and Helleday, T. (2005). Methyl methanesulfonate (MMS) produces heat-labile DNA damage but no detectable in vivo DNA double-strand breaks. Nucleic Acids Res *33*, 3799-3811.
9. Kobayashi, T., and Ganley, A.R. (2005). Recombination regulation by transcription-induced cohesin dissociation in rDNA repeats. Science *309*, 1581-1584.
10. Kobayashi, T., Heck, D.J., Nomura, M., and Horiuchi, T. (1998). Expansion and contraction of ribosomal DNA repeats in Saccharomyces cerevisiae: requirement of replication fork blocking (Fob1) protein and the role of RNA polymerase I. Genes Dev *12*, 3821-3830.
11. Kegel, A., Betts-Lindroos, H., Kanno, T., Jeppsson, K., Strom, L., Katou, Y., Itoh, T., Shirahige, K., and Sjogren, C. (2011). Chromosome length influences replication-induced topological stress. Nature *471*, 392-396.
12. Schollaert, K.L., Poisson, J.M., Searle, J.S., Schwanekamp, J.A., Tomlinson, C.R., and Sanchez, Y. (2004). A role for Saccharomyces cerevisiae Chk1p in the response to replication blocks. Mol Biol Cell *15*, 4051-4063.
13. Houseley, J., Kotovic, K., El Hage, A., and Tollervey, D. (2007). Trf4 targets ncRNAs from telomeric and rDNA spacer regions and functions in rDNA copy number control. EMBO J *26*, 4996-5006.
14. McKee, A.H., and Kleckner, N. (1997). A general method for identifying recessive diploid-specific mutations in Saccharomyces cerevisiae, its application to the isolation of mutants blocked at intermediate stages of meiotic prophase and characterization of a new gene SAE2. Genetics *146*, 797-816.